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Application of the ^{15}N gas-flux method for measuring in situ N_2 and N_2O fluxes due to denitrification in natural and semi-natural terrestrial ecosystems and comparison with the acetylene inhibition technique

Fotis Sgouridis et al.

Correspondence to: Fotis Sgouridis (f.sgouridis@bristol.ac.uk)

Supplementary Table 1: Change of the soil volumetric water content (VWC) after the addition of the ^{15}N-labelled tracer in each sampling plot in June and August 2013.

Field Sites	Volume of soil water (cm^3)	Added tracer volume (cm^3)	Change in VWC (%)
C-PB 1	5685	200	3.5
C-PB 2	4947	200	4.0
C-PB 3	4362	200	4.6
C-PB 4	4883	200	4.1
C-PB 5	4574	200	4.4
C-UG 1	3170	150	4.7
C-UG 2	3039	150	4.9
C-UG 3	2944	150	5.1
C-UG 4	3373	150	4.4
C-UG 5	2921	150	5.1
R-HL 1	4654	200	4.3
R-HL 5	4010	200	5.0
C-MW 1	1617	50	3.1
C-MW 2	1731	50	2.9
C-MW 3	1708	50	2.9
C-MW 4	1707	50	2.9
C-MW 5	1623	50	3.1
R-DW 1	2823	100	3.5
R-DW 2	2764	100	3.6
R-DW 3	2677	100	3.7
R-DW 4	2790	100	3.6
R-DW 5	2742	100	3.6
C-IG 1	2292	100	4.4
C-IG 2	2240	100	4.5
C-IG 3	2299	100	4.3
C-IG 4	2528	100	4.0
C-IG 5	2315	100	4.3
R-UG 1	2696	100	3.7
R-UG 2	2741	100	3.6
R-UG 3	2678	100	3.7
R-UG 4	2673	100	3.7
R-UG 5	2663	100	3.8
R-IG 1	2381	100	4.2
R-IG 2	2369	100	4.2
R-IG 3	2153	100	4.6
R-IG 4	2490	100	4.0
R-IG 5	2392	100	4.2

Supplementary Table 2: Mineral NO_3^--N, ^{15}N-NO_3^- amendment, the estimated enrichment of the total (ambient + tracer) soil nitrate pool in each field site in the June 2013 and August 2013 campaigns and the annual average soil nitrate pool enrichment in the same sites from 17 campaigns between April 2013 and October 2014.

Field Sites	Ambient NO_3^--N (kg ha^{-1})	^{15}N-NO_3^- application (kg ha^{-1})	Enrichment of total soil NO_3^- pool (^{15}N at %)	Average annual soil NO_3^- pool enrichment (^{15}N at %)
C-PB	0.15	0.07	33.2	12.6
C-UG	0.04	0.03	40.2	15.4
C-MW	6.28	1.02	14.0	12.2
C-IG	3.73	0.87	18.8	10.9
R-HL	1.40	0.03	1.8	13.0
R-DW	1.44	0.21	12.7	10.3
R-UG	0.88	0.22	19.9	11.3
R-IG	0.82	0.44	34.7	11.1

Supplementary Table 3: Comparison between the $^{15}X_N$ calculated from both the N_2 and the N_2O isotope ratio data using equation (2). SE = Standard Error.

	$^{15}X_N$	
Field Sites	From N_2	From N_2O
C-PB 1		
C-PB 2		
C-PB 3		
C-PB 4		
C-PB 5		
C-UG 1		
C-UG 2		
C-UG 3		
C-UG 4		
C-UG 5		
R-HL 1	0.93	0.93
R-HL 5		
Mean OS	0.93	0.93
C-MW 1		
C-MW 2	0.63	0.84
C-MW 3		
C-MW 4	0.58	0.79
C-MW 5		
R-DW 1	0.76	0.71
R-DW 2	0.81	0.86
R-DW 3	0.92	0.86
R-DW 4	0.86	0.86
R-DW 5	0.80	0.82
Mean WL	0.77	0.82
SE	0.05	0.02
C-IG 1	0.93	0.84
C-IG 2		
C-IG 3		
C-IG 4	0.99	0.86
C-IG 5		
R-UG 1	0.66	0.48
R-UG 2	0.71	0.59
R-UG 3	0.72	0.44
R-UG 4	0.44	0.22
R-UG 5	0.83	0.81
R-IG 1	0.97	0.95
R-IG 2		
R-IG 3	0.93	0.78
R-IG 4	0.95	0.88
R-IG 5	0.97	0.90
Mean GL	0.83	0.70
SE	0.05	0.07

Supplementary Table 4: Evaluation of the linearity of the evolved N_2 during field incubation, per sampling plot in each field site. Only those samples that were above the MDC value are used. Linear evolution of N_2 in a constant headspace volume is proven when T2/T1 = 2 and T3/T1 ~ 18-24. T1 = 1 hour, T2 = 2 hours and T3 ~ 18-24 hours of incubation time. Ratios close to the ideal values (± 5%) are highlighted in bold font.

	Evolved N_2 (µg N)				
Field Sites	T1	T2	T3	T2/T1	T3/T1
C-PB 1	2.95		3.02		1.02
C-PB 2	10.92	3.70	47.96	0.34	4.39
C-PB 3	3.40	4.85		1.43	
C-PB 4	8.03	4.29	6.73	0.53	0.84
C-PB 5		4.00	2.56		
C-UG 1					
C-UG 2		4.55			
C-UG 3			5.85		
C-UG 4					
C-UG 5					
R-HL 1		12.26	18.96		
R-HL 5			8.58		
C-MW 1	2.17	39.87	30.48	18.38	14.05
C-MW 2	62.13	70.63	40.32	1.14	0.65
C-MW 3	19.70	23.50	5.22	1.19	0.27
C-MW 4	65.93	38.21	26.94	0.58	0.41
C-MW 5	66.71	26.80	20.47	0.40	0.31
R-DW 1	5.65	1.97	50.49	0.35	8.94
R-DW 2	8.28	5.31	143.70	0.64	**17.35**
R-DW 3	8.98	0.67	72.54	0.07	8.08
R-DW 4	22.27		126.14		5.67
R-DW 5	30.23	9.29	71.12	0.31	2.35
C-IG 1	3.96	12.12	97.06	3.06	**24.48**
C-IG 2	4.33	1.78	61.82	0.41	14.27
C-IG 3	4.37	2.59		0.59	
C-IG 4	2.44	3.78	231.27	1.55	94.61
C-IG 5	7.09	6.72	216.28	0.95	30.50
R-UG 1	28.70	2.43	145.85	0.08	5.08
R-UG 2	11.76	43.22	285.30	3.68	**24.27**
R-UG 3	17.02	3.10	414.67	0.18	**24.36**
R-UG 4	25.05	18.67	479.09	0.75	**19.13**
R-UG 5	16.29	4.88	141.80	0.30	8.70
R-IG 1	12.69		217.82		**17.16**
R-IG 2	19.30		51.79		2.68
R-IG 3	27.87				
R-IG 4	27.19				
R-IG 5			20.41		

Supplementary Table 5: Evaluation of the linearity of the evolved N_2O during field incubation, per sampling plot in each field site. Only those samples that were above the MDC value are used. Linear evolution of N_2O in a constant headspace volume is proven when T2/T1 = 2 and T3/T1 ~ 18-24. T1 = 1 hour, T2 = 2 hours and T3 ~ 18-24 hours of incubation time. Ratios close to the ideal values (± 5%) are highlighted in bold font.

	Evolved N_2O (µg N)				
Field Sites	T1	T2	T3	T2/T1	T3/T1
C-PB 1	0.0038	0.0179	0.0468	4.71	12.35
C-PB 2	0.0079	0.0441	0.1235	5.62	15.73
C-PB 3	0.0009	0.0069	0.1182	7.70	131.48
C-PB 4	0.0015	0.0084	0.1328	5.43	86.02
C-PB 5	0.0006	0.0063	0.2711	11.37	492.23
C-UG 1	0.0002		0.0037		**19.75**
C-UG 2	0.0009	0.0049	0.0156	5.48	**17.42**
C-UG 3			0.0031		
C-UG 4			0.0070		
C-UG 5		0.0042	0.0222		
R-HL 1	0.0009	0.0034	0.0051	3.70	5.45
R-HL 5	0.0005	0.0012		2.58	
C-MW 1	0.003	0.008	0.075	2.89	27.32
C-MW 2	0.083	0.221	2.240	2.65	26.92
C-MW 3	0.004	0.009	0.030	**2.11**	7.25
C-MW 4	0.040	0.099	0.387	2.50	9.74
C-MW 5	0.064	0.123	0.575	**1.91**	8.97
R-DW 1	0.098	0.223	0.157	2.28	1.60
R-DW 2	0.033	0.216	0.480	6.64	14.77
R-DW 3	0.028	0.137	1.072	4.96	38.80
R-DW 4	0.039	0.199	0.429	5.10	10.97
R-DW 5	0.004	0.020	0.146	5.51	40.62
C-IG 1	1.379	4.290	13.873	3.11	10.06
C-IG 2	0.157	0.341	3.020	**2.18**	**19.25**
C-IG 3	0.566	3.614		6.38	
C-IG 4	1.208	5.141	23.968	4.26	**19.84**
C-IG 5	1.857	5.865	20.054	3.16	10.80
R-UG 1	0.044	0.180	0.650	4.10	14.80
R-UG 2	0.008	0.071	4.975	9.29	652.28
R-UG 3	0.013	0.285	16.274	21.15	1205.50
R-UG 4	0.035	0.192	17.373	5.55	502.96
R-UG 5	0.036	0.263	5.960	7.40	167.40
R-IG 1	0.946	5.164	7.357	5.46	7.78
R-IG 2	0.117	1.227	6.160	10.47	52.56
R-IG 3	0.046	0.101	0.038	2.21	0.82
R-IG 4	0.040	0.598		14.97	
R-IG 5	0.364	2.813	1.798	7.73	4.94

Supplementary Table 6: Evaluation of the change in the $N_2O/ (N_2 + N_2O)$ ratio with incubation time. The denitrification product ratio is calculated only where both N_2 and N_2O fluxes are available. SE = Standard Error; n = number of samples per land use type.

	$N_2O/ (N_2 + N_2O)$			
Field Sites	T = 1 hour	T = 2 hours	T = 20 hours	Slope
C-PB 1	0.0013		0.0153	0.001
C-PB 2	0.0007	0.0118	0.0026	0.000
C-PB 3	0.0003	0.0014		0.001
C-PB 4	0.0002	0.0020	0.0193	0.001
C-PB 5		0.0016	0.0958	0.005
C-UG 1				
C-UG 2		0.0011		
C-UG 3			0.0005	
C-UG 4				
C-UG 5				
R-HL 1		0.0003	0.0003	0.000
R-HL 5				0.001
Mean OS	0.001	0.003	0.022	
SE	0.0003	0.0018	0.0151	
n	4.00	6.00	6.00	
C-MW 1	0.001	0.000	0.002	0.000
C-MW 2	0.001	0.003	0.053	0.003
C-MW 3	0.000	0.000	0.006	0.000
C-MW 4	0.001	0.003	0.014	0.001
C-MW 5	0.001	0.005	0.027	0.001
R-DW 1	0.017	0.102	0.003	-0.003
R-DW 2	0.004	0.039	0.003	-0.001
R-DW 3	0.003	0.170	0.015	-0.004
R-DW 4	0.002		0.003	0.000
R-DW 5	0.000	0.002	0.002	0.000
Mean WL	0.003	0.036	0.013	
SE	0.002	0.020	0.005	
n	10.00	9.00	10.00	
C-IG 1	0.258	0.261	0.125	-0.007
C-IG 2	0.035	0.161	0.047	-0.002
C-IG 3	0.115	0.583		0.468
C-IG 4	0.331	0.576	0.094	-0.019
C-IG 5	0.208	0.466	0.085	-0.013
R-UG 1	0.002	0.069	0.004	-0.002
R-UG 2	0.001	0.002	0.017	0.001
R-UG 3	0.001	0.084	0.038	0.000
R-UG 4	0.001	0.010	0.035	0.002
R-UG 5	0.002	0.051	0.040	0.001

R-IG 1	0.069		0.033	-0.002
R-IG 2	0.006		0.106	0.005
R-IG 3	0.002			
R-IG 4	0.001			
R-IG 5			0.081	
Mean GL	0.07	0.23	0.06	
SE	0.03	0.07	0.01	
n	14.00	10.00	12.00	